



US006610322B1

(12) **United States Patent**
Keller et al.

(10) **Patent No.:** **US 6,610,322 B1**
 (45) **Date of Patent:** **Aug. 26, 2003**

(54) **SELF FORMING, THERMODYNAMICALLY STABLE LIPOSOMES AND THEIR APPLICATIONS**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/745,292**

(22) Filed: **Dec. 20, 2000**

(51) **Int. Cl.**⁷ **A61K 9/127**

(52) **U.S. Cl.** **424/450; 428/402.2**

(58) **Field of Search** 424/450, 1.21, 424/9.321, 9.51, 417, 94.3, 31.2; 436/829; 935/54; 428/402.2; 264/4.1, 4.3, 4.6

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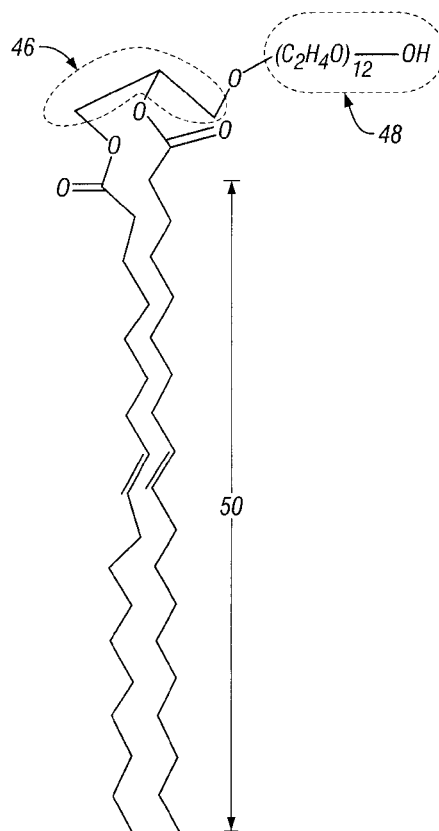
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(57) **ABSTRACT**

A liposome suspension forms spontaneously upon adding a diacylglycerol-PEG lipid to an aqueous solution when the lipid has appropriate packing parameters and the adding occurs above the melting temperature of the lipid. Combinations of lipids may be used in the invention. The liposome suspensions are useful for a variety of purposes, including delivery of therapeutic agents.

17 Claims, 3 Drawing Sheets



PEG-12 Glyceryl Dioleate

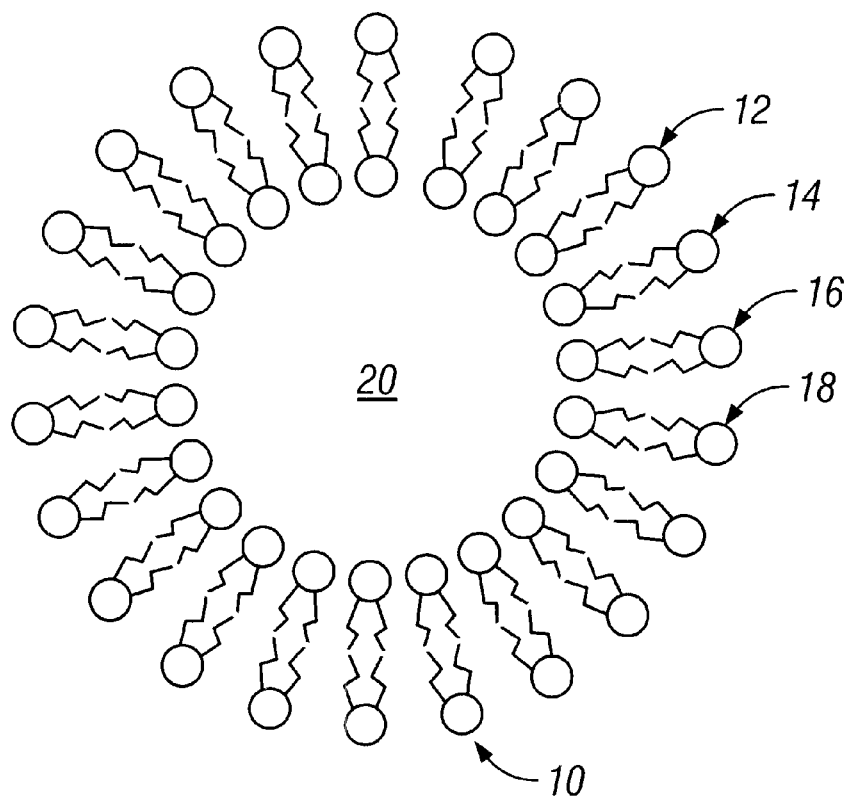


FIG. 1

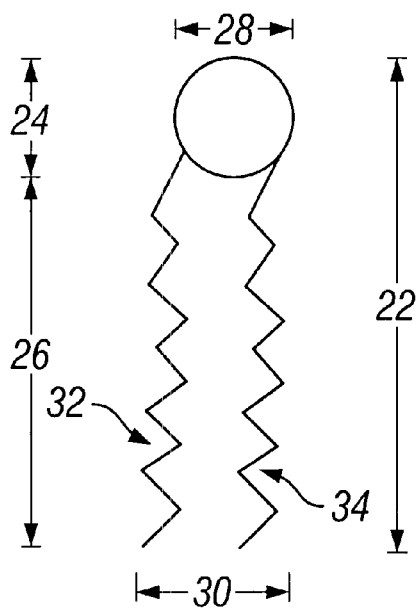


FIG. 2

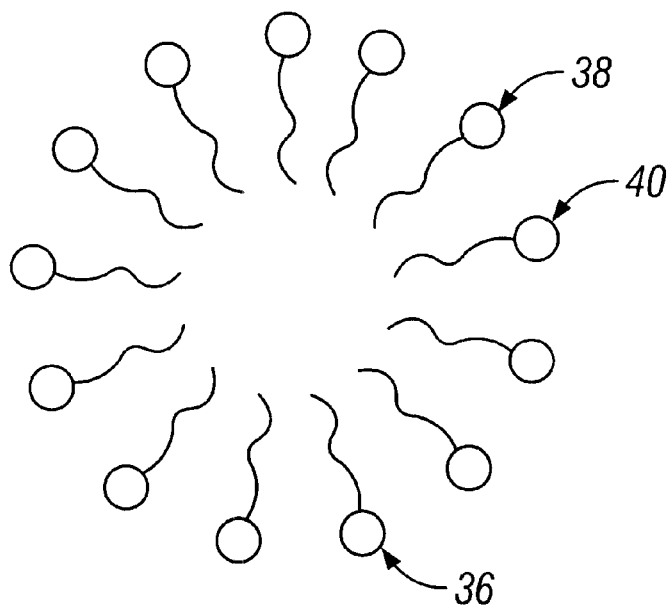


FIG. 3

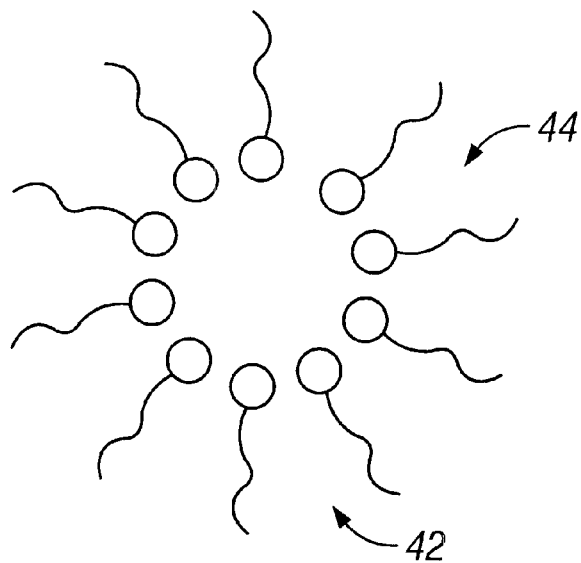
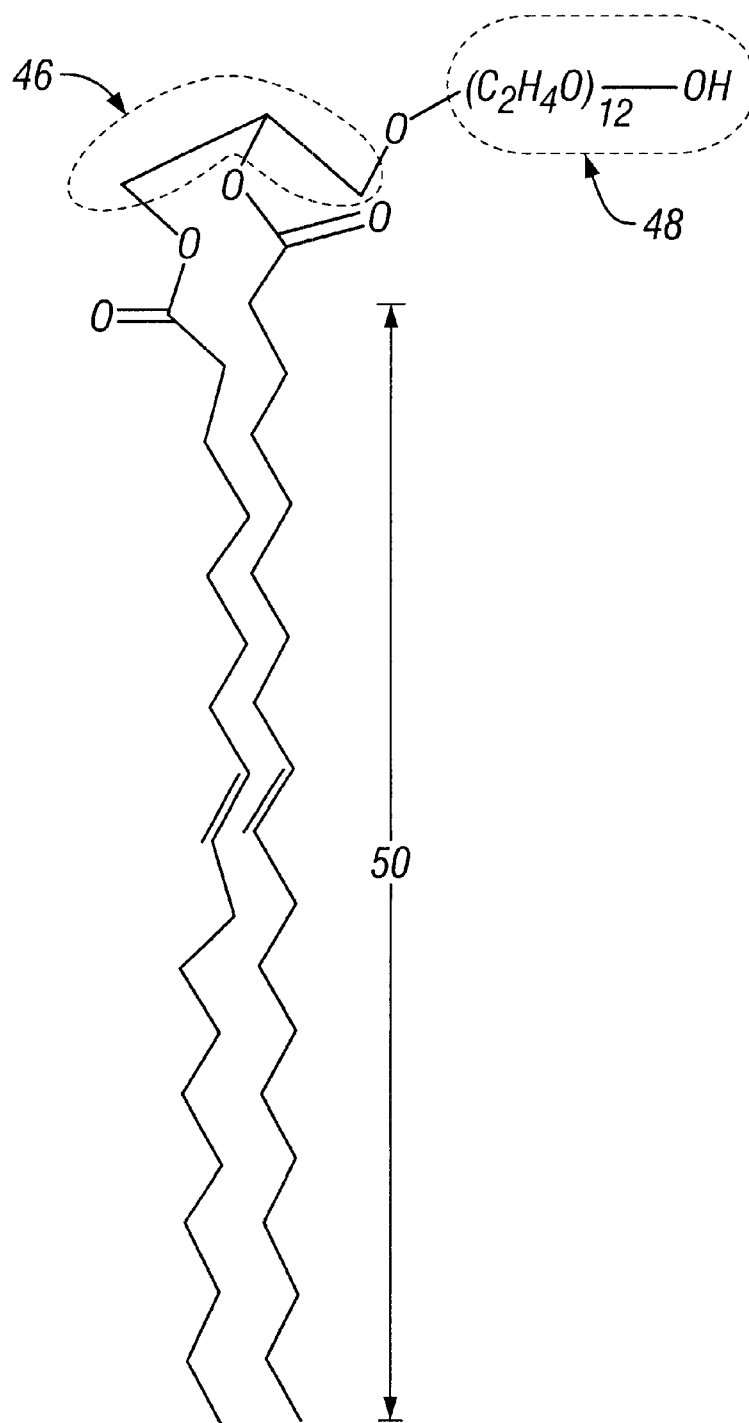


FIG. 4



PEG-12 Glyceryl Dioleate

FIG. 5

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SELF FORMING, THERMODYNAMICALLY STABLE LIPOSOMES AND THEIR APPLICATIONS

FIELD OF THE INVENTION

The present invention relates to liposomes. More particularly, the present invention relates to liposomes which form spontaneously upon mixing of lipids and an aqueous solution, and applications thereof.

BACKGROUND OF THE INVENTION

Liposomes are self-closed colloidal particles in which membranes composed of one or more lipid bilayer(s) encapsulate a fraction of the aqueous solution in which they are suspended. The surfaces of bilayers are hydrophilic while the interior of bilayers, which contain hydrocarbon chains, are hydrophobic. Because of the different microenvironments in their structure, liposomes can encapsulate hydrophilic molecules, bind molecules on the bilayer surfaces or dissolve hydrophobic molecules into the middle of the bilayer. Their ability to incorporate many types of molecules has resulted in applications for drug delivery, diagnostics, cosmetics, cosmeceuticals and nutraceuticals.

Liposomes are manufactured by several different methods. Typically, the process begins when a lipid or combination of lipids are dissolved in an organic solvent. Upon removal of organic solvents and hydration, large multilamellar vesicles (MLVs) are formed. For some applications, small unilamellar vesicles (SUVs) may be desired. SUVs can be produced from MLVs by several techniques including sonication, extrusion through membranes with well-defined pores, French press extrusion and homogenization.

Problems associated with liposomes include colloidal instability, difficulty in scale-up sterilization, and variability between batches in manufacturing. Liposome preparation and manufacturing typically involves removal of organic solvents followed by extrusion or homogenization. These processes may expose liposomal components to extreme conditions such as elevated pressures, elevated temperatures and high shear conditions which can degrade lipids and other molecules incorporated into the liposomes.

Liposome preparations are often characterized by very heterogeneous distributions of sizes and number of bilayers. Conditions optimized on a small scale normally do not scale up well and preparation of large-scale batches is cumbersome and labor intensive.

Another issue associated with liposomes for medical uses is sterilization. Among heat sterilization, ethanol oxide exposure, gamma irradiation and sterile filtration, only the last technique is suitable for liposomes and then only for liposomes smaller than about 100 nanometers (nm). Filtration of liposomes poses many difficulties.

Another problem for liposome applications is colloidal stability. Liposomes in suspension can aggregate and fuse upon storage, heating and addition of various additives. Because of these stability problems, liposomes are often lyophilized. Lyophilization is costly and time consuming. Upon reconstitution, size distributions often increase and encapsulated materials may leak out from the liposomes.

It is therefore desirable to develop new methods and materials which address these problems with current liposome formulations.

BRIEF DESCRIPTION OF THE INVENTION

A liposome suspension forms spontaneously upon adding a lipid composition to an aqueous solution. The lipid com-

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position comprises a single lipid or a mixture of lipids that have appropriate packing parameters, that includes polyethyleneglycol, and that has a melting temperature which allows it to be in liquid form when mixed with the aqueous solution. Such liposome suspensions are useful for a variety of purposes, including the delivery of therapeutic agents.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated into and constitute a part of this specification, illustrate one or more embodiments of the present invention and, together with the detailed description, serve to explain the principles and implementations of the invention.

In the drawings:

FIG. 1 is a diagram depicting the cross-section of a liposome made of lipid molecules.

FIG. 2 is a space-filling diagram of a lipid molecule having a polar head group and nonpolar hydrocarbon chains.

FIG. 3 is a diagram depicting a cross-section of a micelle made of lipid molecules.

FIG. 4 is a diagram depicting a cross-section of a structure made of lipid molecules with large tails relative to the head groups.

FIG. 5 is a diagram showing the molecular structure of PEG-12 Glyceryl Dioleate or Hetoxamate GDO-12.

DETAILED DESCRIPTION

Embodiments of the present invention are described herein in the context of a self-forming, thermodynamically stable liposomes and their applications. Those of ordinary skill in the art will realize that the following detailed description of the present invention is illustrative only and is not intended to be in any way limiting. Other embodiments of the present invention will readily suggest themselves to such skilled persons having the benefit of this disclosure. Reference will now be made in detail to implementations of the present invention as illustrated in the accompanying drawings. The same reference indicators will be used throughout the drawings and the following detailed description to refer to the same or like parts.

In the interest of clarity, not all of the routine features of the implementations described herein are shown and described. It will, of course, be appreciated that in the development of any such actual implementation, numerous implementation-specific decisions must be made in order to achieve the developer's specific goals, such as compliance with application- and business-related constraints, and that these specific goals will vary from one implementation to another and from one developer to another. Moreover, it will be appreciated that such a development effort might be complex and time-consuming, but would nevertheless be a routine undertaking of engineering for those of ordinary skill in the art having the benefit of this disclosure.

Those of ordinary skill in the art will realize that the following description of the present invention is illustrative only and not in any way limiting. Other embodiments of the invention will readily suggest themselves to such skilled persons having the benefit of this disclosure.

Most, if not all, known liposome suspensions are not thermodynamically stable. Instead, the liposomes in known suspensions are kinetically trapped into higher energy states by the energy used in their formation. Energy may be provided as heat, sonication, extrusion, or homogenization. Since every high-energy state tries to lower its free energy,

known liposome formulations experience problems with aggregation, fusion, sedimentation and leakage of liposome associated material. A thermodynamically stable liposome formulation which could avoid some of these problems is therefore desirable.

The present invention teaches liposome suspensions which are thermodynamically stable at the temperature of formation. The formulation of such suspensions is achieved by employing a composition of lipids having several fundamental properties. First, the lipid composition must have packing parameters which allow the formation of liposomes. Second, as part of the head group, the lipid should include polyethyleneglycol (PEG) or any polymer of similar properties which sterically stabilizes the liposomes in suspension. Third, the lipid must have a melting temperature which allows it to be in liquid form when mixed with an aqueous solution.

By employing lipid compositions having the desired fundamental properties, little or no energy need be added when mixing the lipid and an aqueous solution to form liposomes. When mixed with water, the lipid molecules disperse and self assemble as the system settles into its natural low free energy state. Depending on the lipids used, the lowest free energy state may include small unilamellar vesicle (SUV) liposomes, multilamellar vesicle (MLV) liposomes, or a combination of SUVs and MLVs.

Lipid compositions suitable for use in the invention may include compositions comprising only a single type of lipid molecule as well as compositions made up of more than one lipid. As will be appreciated by those skilled in the art, both types of compositions may be quantified according to cited fundamental properties.

One required fundamental property is the ability to form liposomes by virtue of having the proper packing parameters. Packing parameters are relative measures of a given lipid composition, and depend on factors such as size relationships between lipid head groups and lipid hydrocarbon chains, charge, and the presence of stabilizers such as cholesterol. (Israelachvili, D D Lasic, *Liposomes: From Physics to Applications*, Elsevier, pp 51, 1993.)

To form a lipid bilayer, lipid head groups and hydrocarbon chains must organize themselves so that the radius of curvature results in a liposome (see FIG. 1). If the hydrocarbon chains are too small relative to the head group, the radius of curvature will be too large and micelles will be produced (see FIG. 3). If the hydrocarbon chains are too large relative to the head groups, the radius of curvature will be of the opposite sign and liposomes cannot form (see FIG. 4).

FIG. 1 is a diagram depicting the cross-section of a liposome made of lipid molecules. Liposome 10 comprises a lipid bilayer, made of lipid molecules (e.g., 12, 14, 16, 18), enclosing an aqueous space 20.

FIG. 2 is a space-filling diagram of a lipid molecule having a polar head group and nonpolar hydrocarbon chains. Lipid molecule 22 is comprised of a hydrophilic group and a hydrophobic tail 26. Hydrophobic tail 26 may comprise two hydrocarbon chains 32, 34. While its chemical bonds allow the lipid molecule to be flexible, the head group generally fills an area of diameter 28 while the tail fills an area of diameter 30. Because lipid molecules must be organized in a bilayer to form a liposome, the ratio of the head group diameter to the tail diameter can be neither too large nor too small if liposome formation is to occur.

FIG. 3 is a diagram depicting a cross-section of a micelle made of lipid molecules. Micelle 36 is composed of lipid

molecules (e.g., 38, 40). Because the tail groups of the lipid molecules have small diameters relative to the head groups, the lipid molecules organize with a small radius of curvature, and a bilayer cannot form.

FIG. 4 is a diagram depicting a cross-section of a structure made of lipid molecules with large tails relative to the head groups. In FIG. 4, it can be seen that structure 42 forms when lipids (e.g., 44) having large tails relative to the head groups are mixed in aqueous solution. Again, the size ratio between head groups and tails makes bilayer formation impossible.

While FIGS. 1, 3 and 4 have illustrated the basic principle of packing parameters using a single type of lipid molecule, it will be appreciated that the same principle applies to mixtures of lipids. For example, a lipid which has hydrocarbon chains too small to form liposomes as a single species can be mixed with cholesterol to result in a composition which has the proper packing parameters. As another example, a lipid which by itself has the proper packing parameters may form liposomes incorporating limited amounts of other lipids which, by themselves, do not have proper packing parameters. Both single lipids and mixtures of lipids have packing parameters that may be calculated by known methods. In general, liposome compositions which allow liposome formation have packing parameter measurements of P_a between about 0.84 and 0.88 and P_v between about 0.88 and 0.93.

P_a is the packing parameter with respect to surface and P_v is packing parameter with respect to volume (D D Lasic, *Liposomes: From Physics to Applications*, Elsevier, pp 51, 1993). The parameters are derived from the equations $HC_a/T_a = P_a$ and $HC_v/T_v = P_v$, where HC_a is the hydrocarbon chain area, T_a is the total area of the molecule, HC_v is the volume of the hydrocarbon chains and T_v is the volume of the whole molecule.

Packing parameters can be calculated for mixtures of lipids, since ideal mixing of lipids results in arithmetic average of their individual characteristics. For instance $HC_a/T_a = P_a$ of a binary mixture, in the case of ideal mixing can be expressed as:

$$\langle P_a \rangle = X_1 P_{a1} + X_2 P_{a2}, \quad X_1 + X_2 = 1$$

More generally in the case of i lipids composing a given mixture can be represented by:

$$\langle P_a \rangle = \sum_i X_i P_{ai} \quad \text{and} \quad \sum_i X_i = 1$$

where X_i is the mole fraction of the lipid in the mixture and P_i is the packing parameter with respect to surface of that lipid.

FIG. 5 is a diagram showing the molecular structure of PEG-12 Glyceryl Dioleate or Hetoxamate GDO-12 which includes a glycerol backbone 46, a PEG chain 48, and a tail group 50 having two hydrocarbon chains. Together, the backbone 46 and the PEG chain 48 comprise the head group of the molecule. PEG-12 GDO is an preferred lipid molecule for use in the present invention, in part because its head group has the proper size in relation to its tail group.

Two general factors influence the size of the head group in a lipid molecule. One is the actual physical size of the head group. For example, employing a longer PEG chain would make the head group larger. The other is the charge associated with the head group. For example, if the PEG chain was conjugated to the backbone by a phosphodiester bond the phosphate would impart a charge to the head group, effectively increasing its size. In the present invention, non-phospholipids are preferred so that the general means of

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varying head group size is by varying the length of the PEG chain. However, those skilled in the art will recognize that the effective size of the head group could be varied in other ways, for example by using a different backbone other than glycerol or adding a linker such as phosphate between the glycerol backbone and the PEG chain.

The size of the tail of a lipid is mostly influenced by the length of hydrocarbon chains and degree of saturation in the lipid chain. Single chain lipids will generally not form

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Lipids were tested at 2 weight percent in aqueous solution. Note that GDL means glycerol dilaurate, GDO means glycerol dioleate, GDM means glycerol dimyristate, GDP means glycerol dipalmitate, and GDS means glycerol distearate. For each lipid, the number after "PEG" indicates the numbers of C₂H₄O subunits in the PEG chain. The unsaturated dioleate lipids have similar packing parameters to the saturated dimyristate lipids.

lipid	melting point (° C.)	P _a	P _v	Spontaneous Liposomes at 20°C	Spontaneous Liposomes at 37°C	Spontaneous Liposomes at 60°C
PEG-23 GDL	Fluid @ 25	.829	.869	NO	NO	NO
PEG-12 GDO	Fluid @ 25			YES	YES	YES
PEG-23 GDO	Fluid @ 25			NO	NO	NO
PEG-45 GDO	36.3			NO	NO	NO
PEG-12 GDM	Fluid @ 25	.853	.889	YES	YES	YES
PEG-23 GDM	Fluid @ 25	.837	.875	NO	NO	NO
PEG-45 GDM	33.2	.823	.863	NO	NO	NO
PEG-23 GDP	31.2	.843	.880		YES	YES
PEG-45 GDP	41.8	.828	.867	NO	NO	NO
PEG-12 GDS	40.0	.869	.901	NO	NO	YES
PEG-23 GDS	39.8	.849	.885	NO	NO	YES
PEG-45 GDS	40.8	.830	.870	NO	NO	NO

liposomes, though they may be incorporated into liposomes composed of lipids with two chains. Similarly, lipids with one long chain and one short chain may have relatively small tail sizes. Those skilled in the art will recognize that such lipids may be used to form liposomes in the present invention, especially if sterols such as cholesterol are used to stabilize the bilayer.

A second fundamental property of lipid compositions suitable for use in this invention is that the head group of at least one lipid must include a PEG chain. PEG stabilizes the liposomes by creating a steric barrier at the outer surface of the liposomes. Preferably, the PEG chain have a molecular weight between about 300 Daltons and 5000 Daltons, although those skilled in the art will recognize that differing concentrations of PEG on the liposome surface as well as differing chain lengths may be used to stabilize the liposomes.

A third fundamental lipid property is that the lipid composition must have a melting temperature which allows the composition to be in liquid form when mixed with an aqueous solution. Generally, this means that the lipid composition should have a phase transition temperature of between about 0° C. and 100° C. As with packing parameters, melting temperatures may be determined for mixtures of lipids.

Related to melting point of a lipid is bending elastic modulus. Generally preferred in the present invention are lipids with a bending elastic modulus that allows the lipid to be sufficiently flexible to form liposomes in an aqueous solution without the need for large energy inputs. If the bending elastic modulus is too great, the lipid will be too rigid to spontaneously form liposomes in an aqueous solution. Preferably, the bending elastic modulus is between about 0 kt and 15 kt. More preferably, the bending elastic modulus is between about 1 kt and 10 kt. The bending elastic modulus is largely determined by the backbone. Glycerol provides an ideal backbone for the present invention.

Table 1 shows a number of lipids which have been tested as single lipids for suitability for the present invention.

The table shows that lipids which possess the required properties will spontaneously form liposomes when mixed in an aqueous solution. For example, PEG-12 GDM spontaneously forms liposomes at all temperatures tested since it is a liquid at those temperatures and includes PEG in addition to having packing parameters within the required ranges. Similarly, PEG-12 GDO, which shares nearly identical properties to PEG-12 GDM, spontaneously forms liposomes at all temperatures tested.

PEG-12 GDS shows one example of the requirement that the lipid be liquid at the temperature of liposome formation. While this lipid has the required packing parameters as well as including PEG, it does not spontaneously form liposomes until the temperature of liposome formation is high enough for the lipid to be in liquid form.

The GDM series of lipids illustrates the importance of proper packing parameters. While these lipids all include PEG and are in liquid form at 60 degrees, only PEG-12 GDM has the proper packing parameters to allow spontaneous liposome formation. The GDS lipid series at 60 degrees illustrates the same point.

Those skilled in the art can practice the present invention by using knowledge of the required properties to predict and create lipid compositions which will spontaneously form liposomes. For example, certain PEG lipids which form micelles can form liposomes in mixtures with sterols, such as cholesterol, because of cholesterol's effect on packing parameters and melting point. For instance, the mixture of PEG-45 GDS and cholesterol forms liposomes. Similarly, the size of the head group may be changed to affect packing parameters, for example by varying the size of the PEG chain or by varying the concentration of PEG-containing lipids in the lipid composition.

It will be appreciated that, while liposomes form spontaneously at the temperature of formation, cooling of liposomes after formation results in liposomes in trapped kinetic states. To minimize distortions in liposome structure upon such cooling, sterols such as cholesterol may be mixed with the lipid before liposome formation. It has been observed that cholesterol may be dissolved in PEG-12 GDO at up to about 10 percent by weight.

The present invention is useful in a variety of situations, and provides advantages over the prior art in several different ways. Problems with liposome preparation, reproducibility, colloidal stability, sterilization, and storage may be reduced by employing the invention.

Since the liposomes of the present invention are self-forming, liposome preparation entails merely mixing the lipid with an aqueous solution. In general, liposome formation is scale dependant. It is simple, in the case of the present invention, to scale up from test batches to large batches.

Because the liposomes of the present invention exist in the lowest energy state that the lipid can exist in while in aqueous solution, reproducibility of liposome formation is no problem. A defined lipid, lipid mixture, or lipid/compound mixture will form similar liposomes every time when mixed with the same aqueous solution. It should be noted, however, that above critical concentrations (around 20% weight to volume for most lipids) non-liposomal structures will begin to form in aqueous solution.

Aggregation and fusion may occur with liposomes in thermodynamically trapped states. Because the liposomes of the present invention are in the lowest energy state, they do not aggregate and fuse.

Because the liposomes of the present invention are small, they can be sterile filtered. Also, the lipids may be heat sterilized prior to liposome formation.

As with any liposome dispersion, these liposomes can be lyophilized in the presence of appropriate cryoprotectants. Even in liquid form, the liposomes are colloidally stable because they are a thermodynamically stable system. Also, because of their self-forming nature, the liposomes need not be stored at all. Instead, the lipid may be stored and constituted into liposomes as needed.

Applications of the present liposomes include the delivery of therapeutics and other compounds, use in cosmetics, and use in drug delivery screening.

Since the liposomes include an aqueous space, a hydrophobic region within the bilayer, and sites for covalent attachment (e.g., on the PEG chain or the backbone), many types of compounds may be encapsulated by the liposomes. Such compounds include compounds ranging from hydrophilic to hydrophobic, including many insoluble compounds. These liposomes may substitute for currently available Cremophor® and Solutol®.

For delivery of intravenous drugs, the drug may be provided in a sealed container co-dissolved or co-mixed along with the lipid. The container may also contain inert gas to reduce decomposition of the drug and lipid. Prior to administration an aqueous solution is added to the container, thereby forming liposomes with encapsulated drug. Drugs suitable for such a mode of administration include: proteins, peptides, nucleic acids, antineoplastic agents, anti-inflammatories, anti-infectives, gastrointestinal agents, biological and immunologic agents, dermatologic agents, ophthalmic and otic agents, diagnostic aids, nutrients and nutritional agents, hematological agents, endocrine and metabolic agents, cardiovasculars, renal and genitourinary agents, respiratory agents, central nervous system agents.

In one aspect, the invention includes a method of preparing liposomes. The method comprises providing an aqueous solution; providing a lipid solution, where the solution has a P_a between about 0.84 and 0.88, a P_v between about 0.88 and 0.93, and where at least one lipid in the solution includes a polyethyleneglycol (PEG) chain; and combining the lipid

solution and the aqueous solution. The PEG chain preferably has a molecular weight between about 300 Daltons and 5000 Daltons. Kinetic energy, such as shaking or vortexing, may be provided to the lipid solution and the aqueous solution.

The lipid solution may comprise a single lipid. The lipid may comprise dioleoylglycerol-PEG-12, either alone or as one of the lipids in a mixture. The method may further comprise providing an active compound; and combining the active compound with the lipid solution and the aqueous solution.

The active compound may be selected from the group comprising proteins, peptides, nucleic acids, antineoplastic agents, anti-inflammatories, anti-infectives, gastrointestinal agents, biological and immunologic agents, dermatologic agents, ophthalmic and otic agents, diagnostic fluids, nutrients and nutritional agents, hematological agents, endocrine and metabolic agents, cardiovasculars, renal and genitourinary agents, respiratory agents, central nervous system agents.

In another aspect, the invention includes a liposome suspension. The suspension comprises one or more lipids, where the lipids as an aggregate have a P_a between about 0.84 and 0.88, a P_v between about 0.88 and 0.93 and a melting temperature of between about 0 to 100 degrees centigrade; and where at least one lipid includes a polyethyleneglycol (PEG) chain. The PEG chain preferably has a molecular weight between about 300 Daltons and 5000 Daltons. The suspension may comprise a single lipid. The lipid may comprise dioleoylglycerol-PEG-12. The suspension may further comprise an active compound, which may be selected from the group described above.

In another aspect, the invention includes a composition for combining with an aqueous solution to form a liposome suspension. The composition comprises one or more lipids, where the lipids as an aggregate have a P_a between about 0.84 and 0.88, a P_v between about 0.88 and 0.93 and a melting temperature of between about 0 to 100 degrees centigrade; and where at least one lipid includes a polyethyleneglycol (PEG) chain. The PEG chain preferably has a molecular weight between about 300 Daltons and 5000 Daltons. The composition may comprise a single lipid. The composition may comprise dioleoylglycerol-PEG 12. The composition may further comprise an active compound selected from the group above. The composition may be provided in a sealed container, where the container also contains an inert gas to prevent oxidative degradation.

In another aspect, the invention includes a method of intravenously administering a therapeutic compound. The method comprises providing a composition including one or more lipids, where the lipids as an aggregate have a P_a between about 0.84 and 0.88, a P_v between about 0.88 and 0.93 and a melting temperature of between about 0 to 100 degrees centigrade; and where at least one lipid includes a polyethyleneglycol (PEG) chain; providing an active compound; providing an aqueous solution; combining the composition, compound and solution to form a liposome suspension; and administering the liposome suspension intravenously. The method may further comprise providing kinetic energy to the liposome suspension. The method may also include providing the composition in a sealed container containing an inert gas. The PEG chain preferably has a molecular weight between about 300 Daltons and 5000 Daltons. The composition may comprise a single lipid. The lipid may comprise dioleoylglycerol-PEG-12. The active compound may be selected from the group above.

In another aspect, the invention includes a method of solubilizing an active compound. The method comprises

providing a composition including one or more lipids, where the lipids as an aggregate have a P_a between about 0.84 and 0.88, a P_v between about 0.88 and 0.93 and a melting temperature of between about 0 to 100 degrees centigrade; and where at least one lipid includes a polyethyleneglycol (PEG) chain; providing the active compound; providing an aqueous solution; and combining the active compound, the lipid and the aqueous solution to form a liposome suspension. The method may further comprise providing kinetic energy to the liposome suspension. The method may include providing the composition in a sealed container containing an inert gas. The PEG chain preferably has a molecular weight between about 300 Daltons and 5000 Daltons. The composition may comprise a single lipid. The lipid may comprise dioleolglycerol-PEG-12. The active compound may be selected from the group above.

In another aspect, the invention includes a method of solubilizing an active compound. The method comprises providing a composition including one or more lipids, where the lipids as an aggregate have a P_a between about 0.84 and 0.88, a P_v between about 0.88 and 0.93 and a melting temperature of between about 0 to 100 degrees centigrade; and where at least one lipid includes a polyethyleneglycol (PEG) chain; providing the active compound; providing an aqueous solution; and combining the active compound, the lipid and the aqueous solution to form a liposome suspension. The method may further comprise providing kinetic energy to the liposome suspension. The method may include providing the composition in a sealed container containing an inert gas. The PEG chain preferably has a molecular weight between about 300 Daltons and 5000 Daltons. The composition may comprise a single lipid. The lipid may comprise dioleolglycerol-PEG-12. The active compound may be selected from the group above.

The compositions may be administered topically, inter- orally, vaginally or rectally.

While embodiments and applications of this invention have been shown and described, it would be apparent to those skilled in the art having the benefit of this disclosure that many more modifications than mentioned above are possible without departing from the inventive concepts herein. The invention, therefore, is not to be restricted except in the spirit of the appended claims.

EXAMPLES

Example 1

Spontaneous Liposomes

Single lipids were tested for their ability to spontaneously form liposomes when mixed with an aqueous solution. PEG-12 GDO was obtained from Global 7 (New Jersey). All other lipids were synthesized for the experiment. For each experiment, water and lipid were separately equilibrated at the appropriate temperature. Two weight percent lipid was added to the aqueous solution, and the solution was analyzed for the presence of liposomes by optical light microscopy, cryo TEM, freeze fracture TEM, or hot stage microscopy. The results of the experiments are shown in Table 1 above.

Example 2

Formation of Multilamellar Liposomes with Cholesterol

Cholesterol, 10 Wt. %, was added to PEG-12 Glyceryl Dioleate and heated to about 60°–70° C., for 15–20 minutes. Water, at room temperature, was added to the heated lipid solution. The mixture was left overnight. Examination of the preparation under a optical microscope with polarized light

at 100 power and 600 power showed multilamellar liposomes in the size range of 20 nm to 40 nm. No crystals of cholesterol were observed.

Example 3

Spontaneous Liposomes for Intravenously Administering Therapeutic Compounds

Ingredient	Conc. (% wt)
Methotrexate	5
PEG-12 GDO	10
DI Water	85

Methotrexate (Sigma) was dissolved in PEG-12 Glyceryl Dioleate and gently mixed for about 5 minutes. The resultant mixture was a clear solution. Deionized water was slowly added to the solution and gently mixed. The resultant mixture was a yellow, opaque solution. The preparation was examined under a light microscope with polarized light and showed a suspension of liposomes.

Example 4

Spontaneous Liposomes for Intravenously Administered Therapeutic Compound and as a Drug Solubilization Vehicle.

Ingredient	Conc. (wt %)
Cyclosporine A	0.77
PEG-12 GDO	4.30
DI Water	95.23

Cyclosporin A (Sigma 49H4066) was mixed with PEG-12 Glyceryl Dioleate by vortexing and sonication for 10 minutes. Water was added and gently mixed. Examination under optical microscope at 600 power showed multilamellar liposomes and crystals of cyclosporin A.

Example 5

Spontaneous Liposomes with Active Compounds for Dermatology.

Ingredient	Conc. (wt)
PEG-12 GDO	18 g
Betamethasone dipropionate	50 mg
Cholesterol	100 mg
Uniphen-23®	1.5 mg
Water	80.35 g

Weighed amounts of PEG-12 Glyceryl Dioleate, Betamethasone dipropionate and cholesterol were combined and heated to 50° C. while mixing. Uniphen-23® and water were combined and heated to 50° C. When mixtures reached temperature they were commingled while stirring gently. Mixture was cooled to room temperature while stirring. Examination by optical microscope at 100× and 600× showed a suspension of multilamellar liposomes.

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Example 6

Spontaneous Liposomes with Active Compounds for Topical Anesthesia.

Ingredient	Conc.
Tetracaine	2 g
PEG-12 GDO	20 g
Uniphen-23 ®	1.5 g
Water	76.5 g

Tetracaine, PEG-12 Glyceryl Dioleate, and Uniphen-23® were mixed together and heated to 40° C. while stirring. Water was heated to 40 degrees C. and added to the tetracaine solution while stirring gently. Mixture was cooled to room temperature. Examination by electron microscope showed LUV's and MLV's.

Example 7

Spontaneous Liposomes for Intravenous and Topical Formulations

Tretinoin (all-trans retinoic acid), 6 mg, was dissolved in 500 ul of PEG-12 Glyceryl Dioleate. Dissolution was complete. Distilled water, 4.5 ml, was added to the mixture and gently mixed. This yielded a concentration of 1 mg/ml. Examination by optical microscope showed multilamellar liposomes in the size range of 100 nm to 200 nm. This solution can easily be incorporated into a cream, gel or lotion dosage form.

While embodiments and applications of this invention have been shown and described, it would be apparent to those skilled in the art having the benefit of this disclosure that many more modifications than mentioned above are possible without departing from the inventive concepts herein. The invention, therefore, is not to be restricted except in the spirit of the appended claims.

What is claimed is:

1. A method of spontaneously preparing liposomes, the method consisting essentially of:

providing an aqueous solution;

providing one or more diacylglycerol-PEG lipids where the lipid or lipids have a P_a between about 0.84 and 0.88 and a P_v between about 0.88 and 0.93 and where P_a is the packing parameter with respect to surface and P_v is the packing parameter with respect to volume; and combining the lipid or lipids and the aqueous solution at a temperature above the melting point of the lipid or lipids.

2. The method of claim 1, where the PEG chain of the lipid or lipids has a molecular weight between about 300 Daltons and 5000 Daltons.

3. The method of claim 1, where the diacylglycerol-PEG lipid comprises dioleoylglycerol-PEG-12.

4. The method of claim 1, further comprising:

providing an active compound; and

combining the active compound with the lipid or lipids and the aqueous solution.

5. The method of claim 4, where the active compound is selected from the group consisting of proteins, peptides, nucleic acids, agents for treating neoplasms, agents for treating inflammation, agents for treating infections, agents for treating gastrointestinal diseases, agents for treating immunological diseases, agents for treating skin diseases, agents for treating eye diseases, agents used in diagnosing disease, nutrients, agents for treating blood diseases, agents for treating metabolic diseases, agents for treating cardio-

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vascular disease, agents for treating renal diseases, agents for treating genitourinary diseases, agents for treating respiratory diseases, and agents for treating central nervous system diseases.

6. A method of intravenously administering a therapeutic compound, the method consisting essentially of:

providing one or more diacylglycerol-PEG lipids where the lipid or lipids have a P_a between about 0.84 and 0.88 and a P_v between about 0.88 and 0.93 and where P_a is the packing parameter with respect to surface and P_v is the packing parameter with respect to volume;

providing the therapeutic compound;

providing an aqueous solution;

combining the lipid or lipids, compound and solution at a temperature above the melting point of the lipid or lipids to spontaneously form a liposome suspension; and

administering the liposome suspension intravenously.

7. The method of claim 6, where the PEG chain of the lipid or lipids has a molecular weight between about 300 Daltons and 5000 Daltons.

8. The method of claim 6, where the diacylglycerol-PEG lipid comprises dioleoylglycerol-PEG-12.

9. The method of claim 6, where the therapeutic compound is selected from the group consisting of proteins, peptides, nucleic acids, agents for treating neoplasms, agents for treating inflammation, agents for treating infections, agents for treating gastrointestinal diseases, agents for treating immunological diseases, agents for treating skin diseases, agents for treating eye diseases, agents used in diagnosing disease, nutrients, agents for treating blood diseases, agents for treating metabolic diseases, agents for treating cardiovascular disease, agents for treating renal diseases, agents for treating genitourinary diseases, agents for treating respiratory diseases, and agents for treating central nervous system diseases.

10. A method of solubilizing an active compound, the method comprising:

providing one or more diacylglycerol-PEG lipids where the lipid or lipids have a P_a between about 0.84 and 0.88 and a P_v between about 0.88 and 0.93 where P_a is the packing parameter with respect to surface and P_v is the packing parameter with respect to volume;

providing the active compound;

providing an aqueous solution; and

combining the active compound, the lipid or lipids and the aqueous solution to spontaneously form a liposome suspension, where said combining occurs at a temperature above the melting point of the lipid composition.

11. The method of claim 10, where said providing one or more diacylglycerol-PEG lipids includes providing the lipid or lipids in a sealed glass container containing an inert gas.

12. The method of claim 10, where the PEG chain of the lipid or lipids has a molecular weight between about 300 Daltons and 5000 Daltons.

13. The method of claim 10, where the diacylglycerol-PEG lipid comprises dioleoylglycerol-PEG-12.

14. The method of claim 10, where the active compound is selected from the group consisting of proteins, peptides, nucleic acids, agents for treating neoplasms, agents for treating inflammation, agents for treating infections, agents for treating gastrointestinal diseases, agents for treating immunological diseases, agents for treating skin diseases, agents for treating eye diseases, agents used in diagnosing disease, nutrients agents for treating blood diseases, agents for treating metabolic diseases, agents for treating cardio-

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vascular disease, agents for treating renal diseases, agents for treating genitourinary diseases, agents for treating respiratory diseases, and agents for treating central nervous system diseases.

15. A method of orally administering a therapeutic compound, the method comprising:

providing one or more diacylglycerol-PEG lipids where the lipid or lipids have a P_a between about 0.84 and 0.88 and a P_v between about 0.88 and 0.93 where P_a is the packing parameter with respect to surface and P_v is the packing parameter with respect to volume;

providing an active compound;

providing an aqueous solution;

combining the lipid or lipids, compound and solution to form a liposome suspension, where said combining occurs at a temperature above the melting point of the lipid composition; and

administering the liposome suspension orally in the form selected from the group consisting of a two piece hard gelatin capsule, a soft gelatin capsule, and drops.

16. A method of orally or sublingually administering a therapeutic compound, the method comprising:

providing one or more diacylglycerol-PEG lipids where the lipid or lipids have a P_a between about 0.84 and 0.88 and a P_v between about 0.88 and 0.93 where P_a is the packing parameter with respect to surface and P_v is the packing parameter with respect to volume;

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providing the therapeutic compound;

providing an aqueous solution;

combining the lipid or lipids, compound and solution to form a liposome suspension, where said combining occurs at a temperature above the melting point of the lipid composition; and

administering the liposome suspension orally by spraying it into the mouth.

17. A method of administering a therapeutic compound, the method comprising:

providing one or more diacylglycerol-PEG lipids where the lipid or lipids have a P_a between about 0.84 and 0.88 and a P_v between about 0.88 and 0.93 where P_a is the packing parameter with respect to surface and P_v is the packing parameter with respect to volume;

providing the therapeutic compound;

providing an aqueous solution;

combining the lipid or lipids, compound and solution to form a liposome suspension, where said combining occurs at a temperature above the melting point of the lipid composition;

incorporating the suspension into a form selected from the group consisting of a cream, a lotion, and a gel; and administering the liposome suspension.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,610,322 B1
DATED : August 26, 2003
INVENTOR(S) : Brian Charles Keller and Dan D. Lasic

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 3,

Line 39, replace "D D" with -- DD --.

Line 56, after "group" insert -- 24 --.

Column 4,

Line 28, replace "D D" with -- DD --.

Line 45, replace " $\langle P_a \rangle = \sum_i X_i P_i$ and $\sum_i X_i = 1$ " with -- $\langle P_a \rangle = \sum_i X_i P_i$ and $\sum_i X_i = 1$ --.

Column 7,

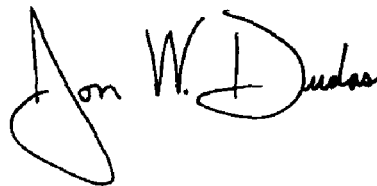
Line 55, replace "dermtaologic agents" with -- dermatologic agents, --.

Column 8,

Line 14, replace "fluids" with -- aids --.

Signed and Sealed this

Ninth Day of March, 2004

A handwritten signature in black ink, reading "Jon W. Dudas". The signature is stylized, with a large loop for the "J" and a distinct "D".

JON W. DUDAS

Acting Director of the United States Patent and Trademark Office

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Column 8,

Line 14, replace "fluids" with -- aids --.

Column 9,

Line 15, replace "dioleolglycerol" with -- dioleolyglycerol --

This certificate supersedes Certificate of Correction issued March 9, 2004.

Signed and Sealed this

Seventh Day of December, 2004

A handwritten signature in black ink, reading "Jon W. Dudas". The signature is stylized, with a large, looped initial "J" and a distinct "D" at the end.

JON W. DUDAS

Director of the United States Patent and Trademark Office